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Assessment of the Impact of Magnesium and Nitrogen Fertilization on Two Species of Grasses Used as Horse Feed

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Abstract: The aim of this study was to determine the effect of nitrogen (three doses of N) and magnesium (two doses of Mg) fertilization on the yield and quality of fodder obtained from two old and extensive grass species *Festulolium braunii* cv. 'Felopa' and *Lolium multiflorum* cv. 'Tur' in field cultivation under dry conditions. *F. braunii* was better adapted to cultivation on light, dry soils than *L. multiflorum*; in such conditions, it produces higher yields of dry matter and protein, characterized by a higher concentration of nutrients. *F. braunii* fertilized with doses of 120 and 180 kg N·ha^{-1} yielded higher than that fertilized with a dose of 60 kg N·ha^{-1}, and *L. multiflorum* produced similar yields after applying doses of 60, 120 and 180 kg N·ha^{-1}. For tested grass pasture, a single N application after the start of vegetation in two forms (fast- and slow-acting) appears to be adequate. Resignation from splitting the nitrogen dose due to variable rainfall distribution that can occur after the first cut during the dry summer is beneficial due to a reduction in the losses of nutrients and environmental burdens.

Keywords: metabolizable energy; mineral nitrogen forms; nutrient use efficiency indices; N uptake; nitrate content; protein; fiber

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1. Introduction

Aggravating effects of climate change, mainly in the form of intensifying periods of drought, increasingly reduce the effects of crop production and generate environmental burdens. They are particularly dangerous in regions with light soils, represented by a limited source of carbon-based compounds, and thus, low water retention. In such conditions, only extensive cultivation of plants is possible, especially grasses that cover the soil for a long time and can also survive a difficult period of drought. In grass cultivation, it is most often recommended to use split nitrogen doses and apply them after each cut. Such a procedure does not work in regions with frequent summer droughts and a low sum of precipitation. In such conditions, the use of nitrogen fertilization after the first cut becomes ineffective because the lack of rainfall prevents the plants from taking up this component. In addition, fertilizers lying on the surface of the field are exposed to weather conditions and can decompose, resulting in nitrogen losses. When using urea in the summer, in conditions of persistent drought, the loss of nitrogen may exceed even 64% and result in low fertilizer efficiency [1]. It is a very hygroscopic fertilizer and even 0.5 mm of precipitation, or fog, can dissolve the granules, initiating transformation processes very quickly [2,3]. Such losses of nitrogen are dangerous in terms of an economic (3.34 million tons of urea = USD 40.5 billion projected losses through volatilization, leaching and denitrification [4]), health (lung diseases caused by NH_3 reaction with acidic compounds in the atmosphere (as $PM_{2.5}$) [5,6]) and environmental (eutrophication [7]) point of view.

In turn, when top dressing with ammonium nitrate, a concentrated fertilizer that acts quickly in dry conditions, which can also be quickly dissolved by a small amount of water from drizzle, fog or dew, in the case of high solar exposure, in addition to possible nitrogen losses, damage is often caused to plants and, in extreme cases, results in situations of the 'burn' of leaves [8] of the sward.

In arid and semi-arid regions of the world the technique of integration of water and fertilizer is a viable way to balance the requirements of water and fertilizer in crop production and to promote their efficiency [9]. A high-efficiency and eco-friendly slow-release fertilizer [10] application reduced nutrient leaching (urease inhibitor decreased the volatilization of NH_3 and N_2O) [11] and cut down the fertilization frequency and dosage, which are helpful to lower the risk of environmental pollution [12]. In tropical pastures, fertilization is advisable in the rainy season and with the use of ammonium sulfate fertilizer as a strategy for lower volatilization losses and higher forage productivity [13]. New reports show that even moderate levels of N additions to dry soils and clay-rich (but also sandy) soils can cause a significant leaching risk when fertilizer is applied during drought conditions. The level and timing of fertilizer applications are the two main factors that influence the leaching of nitrate from arable land [14]. It should be pointed out that the problem is that the interactions of grassland management, especially with fertilization, which can go up to rates of 300 kg nitrogen per hectare per year [15] with climate change extremes, have received only limited attention.

One of the most important goals of modern agriculture in the context of growing pressure on the environment is improving the use of nutrients from fertilizers [16]. the strategy in grassland management that we assumed is, apart from balanced N fertilization, the possibility of improving the use of N by plants by providing them with Mg nutrition, as a way to increase nitrogen fertilizer efficiency, which signals the future strategy of improving fertilizer use efficiency as a key to sustainable agricultural production. The literature shows that some crop plants are well supplied with Mg since the beginning of their growth increases N uptake, resulting in an increase in their unit productivity [17,18]. The yield-forming effect of the applied Mg on grass has not yet been well recognized, which becomes particularly important in a situation where this nutrient is treated as forgotten [17]. The latest report indicated that high-temperature stress caused a decrease in Rubisco carboxylation activity, which inhibited photosynthesis during, for example, the wheat senescence stage. However, the newest research showed that Mg application maintained Rubisco carboxylation by enhancing its activation state and stabilizing the electron transfer rate. Shao et al. [19] assumed that Mg application sustained photosynthesis under high-temperature conditions.

To evaluate different fertilization strategies for grass yields, it is important to study the environmental effects of such fertilization to contribute to the development of more sustainable systems. Therefore, the objective of this study was to investigate the effect of one dose of Mg (25 kg MgO·ha^{-1}) and three doses of N (60, 120 and 180 kg N·ha^{-1}) on forage accumulation, chemical composition, N uptake by grasses (*Lolium* and *Festulolium* sp.) and its use efficiency under field cultivation in the dry areas of the Greater Poland Voivodship in Poland. Moreover, it was assumed that a one-time application of nitrogen fertilization in the form of nitrate and urea after the start of vegetation allows for the proper supply of grass plants with this macronutrient throughout the entire vegetation period, without causing the accumulation of nitrates in the forage and limiting environmental impacts.

2. Materials and Methods

2.1. Experimental Site

The field study was carried out in a grass pasture, *Festulolium braunii* 'Felopa' and *Lolium multiflorum* 'Tur', established in 2012/2013 and 2013/2014, and located on an individual farm in the village of Kunowo, Duszniki commune, Szamotuły district (52°46′34″ S and 16°44′98″ W). In accordance with the Köppen climatic classification, the climate in the region is 'warm-temperate' with a significant predominance of oceanic influences, i.e., smaller annual temperature amplitudes, early spring and summer, and short winter.

The annual rainfall is 547 mm, and the average air temperature is 9.5 °C [20]. In 1969, Schmuck [21] classified this region of Greater Poland among the warmest regions in terms of temperature thermally, and in terms of precipitation, among the very driest regions, making it the area with the greatest water shortages in agriculture [22]. The soil is typical clay-illuvial soil [23] and Albic Luvisols [24] according to Polish Soil Classification and FAO-WRB, respectively. Humus horizons of these soils are characterized by a texture of loamy sand in line with USDA classification (Soil Survey Division Staff, 1993) [25]. The soil is classified into IVb classes according to Polish bonitation classification and belongs to good rye agricultural land suitability complexes. The soil contained sand 85%, silt 13% and clay 2%, with a pH (KCl) 5.5, P_2O_5 21.2, K_2O 9.9 and Mg 2.6 (mg·100·g soil^{-1}) in 2013 and a pH (KCl) 5.1, P_2O_5 30.0, K_2O 14.1 and Mg 2.7 (mg·100 g soil^{-1}) in 2organic matter content low organic matter content and low capacity for soil water retention. In both years, the soil's phosphorus content was very high, its potassium content was medium and its magnesium content was low.

2.2. Weather Conditions

Meteorological data were measured throughout the experimental period, including temperature and rainfall. Based on the collected data, the hydrothermal index (K) of water protection called the 'coefficient of water assure to plant or moisture balance' were calculated according to Sielianinov (Figure 1). It was used to estimate the intensity of drought periods. To calculate the value of K, the following formula was used: K = (Mo × 10)/(Dt × days) where K is the hydrothermal coefficient for an individual month during the growing season, Mo is total monthly precipitation and Dt is the mean daily temperature in a particular month. The variability in weather conditions in the years of the study is reflected in the values of the index K, which combine temperature and precipitation. Lower values of this index were recorded in April, July and August in 2013 and in June, July and September in 2014.

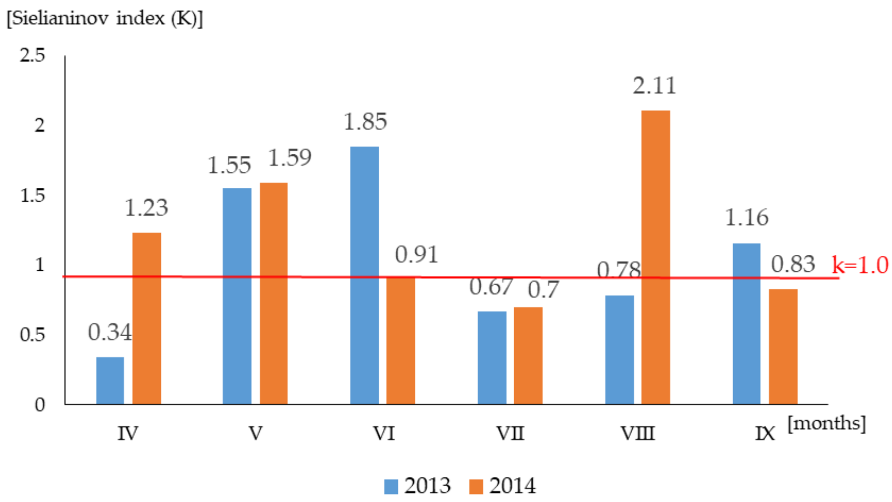


Figure 1. Hydrothermal Sielianinov index (K) in the growing season (April to September) in 2013 and 2014 (Sielianinov index (K): <0.5—drought, 0.5–1.0—semi-drought, 1.0–1.5—border of optimal moisture, >1.5—excessive moisture).

2.3. Experimental Design and Treatments

Two series of two-factor experiments were conducted in parallel, separately with the cultivation of *Festulolium braunii* 'Felopa' variety and *Lolium multiflorum* 'Tur' variety, in a split-plot design, in a 1 + 1 × 3 + 1 factorial scheme, with 4 repetitions. The varieties of both species were old and extensive. The experimental plots were 3 m wide and 20 m long (area of 60 m^2). The entire area covered by the experiment, including isolation zones, measured 1294 ha, including an experimental area of 7680 m^2. The treatments were one dose of soil-one-stage-applied magnesium (Mg) in the form of kieserite: 25 kg MgO·ha^{-1} (15 kg Mg·ha^{-1}) (K_gMg_{ha}), three doses of one-stage-applied nitrogen (N) in the form of ammonium sulphate (20 kg N·ha^{-1}) and urea (40, 100, 160 kg N·ha^{-1}) (K_gN_{ha}): 60 (20 + 40), 120 (20 + 100) and 180 (20 + 160) kg·ha^{-1}, and one treatment without magnesium and nitrogenous fertilization (control). The experimental setup includes the Mg factor: 0 and 15 kg Mg·ha^{-1} and the N factor: 0, 60, 120 and 180 kg Mg·ha^{-1}. The fore-crop in each year of the study was winter barley. A pre-sowing fertilizer with phosphorus and potassium was applied at 80 kg P_2O_5 ha^{-1} (34.9 kg P·ha^{-1}) and 120 kg K_2O (100 kg K·ha^{-1}), respectively.

2.4. Agronomic Management

The stubble disk was used to prepare soil to establish the experiments. After the emergence of volunteer plants, the field was additionally cultivated (Maschio Gaspardo rototiller) (15 cm depth), and after weed emergence, the soil was prepared (Arest cultivator) for sowing (Poznaniak Unia grain seeder) (approximately 1 cm depth with a row spacing of 11 cm), after which a smooth roller was used. Sowing dates were 31 August and 16 September in 2012 and 2013, respectively. In both years, emergence was evenly matched and no winter plant losses were observed. Elite B seeds (Danko® Plant Breeding, Choryń, Poland) of *Festulolium braunii* 'Felopa' variety with 3.77 g TSM and 83% germination capacity and *Lolium multiflorum* 'Tur' variety with 2.34 g TSM and 92% germination capacity were used as seed material for the experiments' establishment. In both experiments, the sowing standard was adopted as 40 kg·ha^{-1}. In the second year, the sowing rate was increased by 5%, i.e., to 42 kg·ha^{-1}. A multi-functional self-propelled mower (Profihopper 1250 SmartLine Amazone®, Amazonen-Werke H. Dreyer SE & Co. KG, Hasbergen, Germany) was used for harvesting without plant loss, with the cutting unit set at a height of 6 cm.

2.5. Dry Matter Yield

To determine the yield, plants were harvested two times (2013: 1st cut 11 June and 2nd 31 July 2014: 1st cut 3 June and 2nd 24 September) and the obtained biomass was weighed with a scale (Radwag WPT/4F, Radom, Poland) directly in the field with 0.1 kg accuracy.

At the same time, samples of 0.5 kg of the above-ground parts of grass plants were randomly taken from each plot and in four replicates to determine the dry matter content and perform chemical analyses. The weight of the sampled plant material was included in the total yield from each plot.

2.6. Relative Chlorophyll Content and Leaf Area Index Measurements

Chlorophyll content measurements were made using the SPAD 502 apparatus (Konica-Minolta, Tokyo, Japan). SPAD measurements were performed on 20 randomly selected plants on upper leaf plates of plants in the early flowering phase. The LAI (leaf area index) value was determined with the LAI 2000 apparatus (LI-COR, Lincoln, NE, USA) in 4 repetitions, and four measurements on the canopy in the full flowering phase.

2.7. Chemical Analysis

The measurement of chloroplast pigment content was performed using the direct method and spectrophotometrically determined (Spekol type) at the appropriate wavelength (chlorophyll a/665 nm, chlorophyll b/649 nm). The amount of chlorophyll a (Chl_a) and chlorophyll b (Chl_b), and the sum of chlorophyll a + b (Chl_{a+b}) and carotenoids (Car) were calculated by using the formulas contained in Reference [26].

Acid invertase was assayed according to Copeland and Lea [27] and expressed as absorbance value·g^{-1} of tissue.

The method described by Jaworski [28] was used to determine the nitrate reductase activity expressed as nmole of NO_2·1 g^{-1} of fresh leaf matter·1 h^{-1}.

The concentration of nitrates was determined in the obtained supernatant by the method of Cataldo et al. [29]. Absorbance was measured at 410 nm. The results were expressed as mg of NO_3·g^{-1} of tissue.

The samples were analyzed for K, Ca and Mg in an atomic absorption spectrophotometer (SpectraAA 55B, Varian, Palo Alto, CA, USA). The P content in the extract was measured at 420 nm by spectrophotometer (Specord–200, Analytic Jena AC, Konrad, Germany) following the procedure of Jones and Case [30].

Moreover, the chemical composition analysis included measurements of dry matter content, crude protein and crude fibre content that were carried out according to standard AOAC procedures [31]. The nitrogen content of the plant biomass was determined using the Kjeldahl method [31] and expressed as total protein content (N × 6.25) [31] and crude fat using the Soxhlet method [32]. The crude fibre was determined via the hydrolyzation of other components of the plant material, and crude ash was determined via incineration. Nitrogen-free extracts were calculated by subtracting the percentage of the remaining nutrients from 100%.

The contents of available forms of phosphorus (P) [33] and potassium (K) [34] in the soil samples collected from each experimental plot were defined by the Egner–Riehm method, as was the content of magnesium available to plants (Mg) [35] following the Schachtschabel method.

The assessment of mineral nitrogen in the two soil layers (0–30 cm, 30–60 cm) after every cut was carried out in accordance with the research procedure (OSCHR, Poznań, Poland); N-NH_4—PB.50 ed. 6 from 17 October 2008; N-NO_3—PB.50 ed. 6 from 17 October 2008.

2.8. Parameters and Indices of Nitrogen Use Efficiency

Based on the results of chemical analyses, equations were used to calculate the amount of N and the N use efficiency (NUE) indices, which are presented below.

1. The use of nitrogen per dose of the mineral fertilizer:

$$\mathrm{N}\ (\%) = (\mathrm{Nf} - \mathrm{Nc}) \times 100/\mathrm{D}, \quad (1)$$

where N—use of nitrogen (%), Nf—nitrogen uptake by fertilized plants (kg·ha^{-1}), Nc—nitrogen uptake by plants in the control (unfertilized) plot (kg·ha^{-1}) and D—nitrogen rate (100 kg·ha^{-1}).

2. Agricultural effectiveness:

$$\mathrm{Ae} = (\mathrm{GYN} - \mathrm{GY0})/100, \quad (2)$$

where Ae—agricultural effectiveness (kg dry matter/kg N in fertilizers), GYN—grain yield in the field with a dose of nitrogen (t·ha^{-1}) and GY0—grain yield in the field without nitrogen application (t·ha^{-1}).

3. Physiological effectiveness

$$\mathrm{Pe} = ((\mathrm{GYN} - \mathrm{GY0})/(\mathrm{Nf} - \mathrm{Nc})) \times 100, \quad (3)$$

where Pe—physiological effectiveness (kg dry matter·kg N in fertilizers), GYN—grain yield in the field with a dose of nitrogen (t·ha^{-1}), GY0—grain yield in the field without nitrogen application (t·ha^{-1}), Nf—nitrogen uptake by fertilized plants (kg·ha^{-1}) and Nc—nitrogen uptake by plants in the control (unfertilized) plot (kg·ha^{-1}).

2.9. Metabolizable Energy

Based on the chemical analysis, the metabolizable energy for horses in the dry matter of the tested grass species was calculated. The following formula was used [36]:

$$\mathrm{Em} = -3.54 + 0.0209\ \mathrm{CP} + 0.0420\ \mathrm{CF} + 0.0001\ \mathrm{CFB} + 0.0185\ \mathrm{NFE}, \quad (4)$$

where Em—metabolizable energy (MJ·kg dry matter^{-1}), CP—crude protein (g·kg dry matter^{-1}), CF—crude fat (g·kg dry matter^{-1}), CFB—crude fibre (g·kg dry matter^{-1}), NFE—nitrogen-free extracts (g·kg dry matter^{-1}).

2.10. Statistical Analysis

The collected data were subjected to an analysis of variance using STATISTICA® 13 (StatSoft, Inc., Krakow, Poland, 2013). The effects of the experimental factor Mg and N dose on agronomic parameters, content of nutrients in plant material, forms of N mineral content in soil after harvest, nitrogen accumulation and indices of its efficiency were tested with a two-way ANOVA, separately for the two grass species. Homogeneous subsets of mean were identified by means of Duncan's test, at a significance level of $\alpha = 0.05$. The dependence of the dry matter yield of the tested grass varieties' plants fertilized with nitrogen was determined using linear and polynomial regression. A heat map was proposed as a graphical presentation of appropriately transformed data of all parameters studied. Data transformation using 'normalise' was used to compare and group features that have different units or scales, using a standardization method. Colors were used to denote the levels of fertilizer factor. Tree diagrams were used for grouping by using the Ward Hierarchical Clustering method and the squared Euclidean distance measurement. The relationship between the parameters studied was determined with the Pearson correlation coefficient and presented as a correlation matrix heatmap.

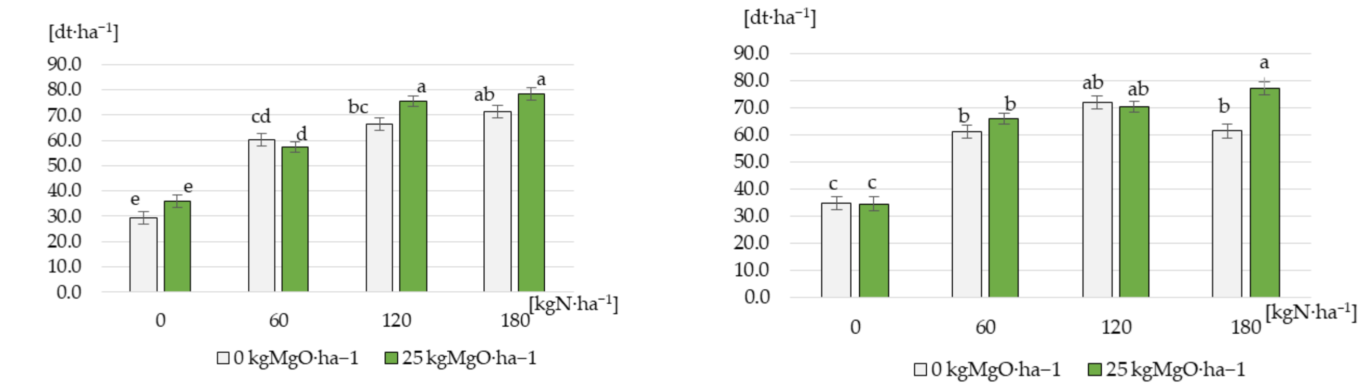
3. Results

3.1. Dry Matter Yield

The yields obtained from *Festulolium braunii* cultivation in the arid conditions of Kunowo were higher by 3.9 dt·ha^{-1} than *Lolium multiflorum* on average, in the years 2013–2014 (Tables S1 and S2). The first cut constituted the main share in the total yield of the studied species in both years (2013, 2014). For *F. braunii* plants, it was 81.8 and 66.1%, respectively, and for *L. multiflorum*, 83.7 and 74.1%.

The synthesis of the results conducted on *F. braunii* 'Felopa' indicated a favorable tendency to increase the total yield of the dry matter of plants after enriching nitrogen fertilization with a dose of 25 K_gMg_{ha}. This regularity in the cultivation of this species, although statistically only proved for the 120 N dose, was observed for all tested N doses, except 60 kg·ha^{-1}. This result was determined by the reaction of *Festulolium* sp. plants harvested in the first cut, which occurred with better water availability, and the yield increase was from 6.7 dt·ha^{-1} when nitrogen fertilization was omitted to 9.1 dt·ha^{-1} after a dose of 120 K_gN_{ha} was used (Figure 2a). However, the reaction of plants to such fertilization in the second half of the growing season, characterized by water shortages, was the opposite, which was manifested in a tendency to a slight decrease in yield, and thus, the effect shown in the synthesis of years was flattened. The analysis of variance showed a significant interaction of nitrogen and magnesium fertilization in influencing the dry matter yield of *L. multiflorum* plants. These interactions resulted in a significant increase in dry matter yield between the nitrogen doses tested; however, the differences were proven only after the use of magnesium for doses of 180 K_gN_{ha}.

Plants of both grass species responded to magnesium fertilization with an increase in the yield of the first cut, except for *L. multiflorum* in 2013. Significant differences in this respect were demonstrated for *F. braunii* and *L. multiflorum* in 2014, and the effect of the introduced fertilization was an increase in dry matter yield by 3.9 and 9.4 dt·ha^{-1}, respectively (Tables S1 and S2). The use of combined nitrogen and magnesium fertilization in the cultivation of *L. multiflorum* confirmed the favorable tendency to increase the yield of dry matter in the first cut shown for *F. braunii* plants, but only for doses of 60 and 180 K_gN_{ha} (Figure 2b). It is worth emphasizing the particularly beneficial effect of introducing 25 kg of MgO with a dose of 180 K_gN_{ha}, which resulted in an increase in the dry matter yield of the first cut amounting to 15.9 dt·ha^{-1}.



(**a**) (**b**)

Figure 2. The dry matter yield of (**a**) *Festulolium braunii* and (**b**) *Lolium multiflorum* plants harvested in the first cut (dt·ha^{-1}) in response to the interaction of Mg and N fertilization; on average in 2013-2014; letters denote homogeneous groups in Duncan's test ($p < 0.05$).

The regression analysis showed that in arid conditions of light soils, the maximum total dry matter yield of *F. braunii* is higher by 9.4 dt·ha^{-1} than *L. multiflorum*, provided that a dose of nitrogen is increased by 49.7 dt·ha^{-1} (Table 1). In both species, the introduction of magnesium fertilization led to an increase in the total dry matter yield and yield harvested in the first cut. In each case, the nitrogen dose had to be increased. A different reaction of plants occurred in the case of the second cut in both grass species, cultivated in conditions of water deficiency, and therefore, nitrogen deficiency, and it was described by straight-line equations. Greater increases in dry matter per 1 kg of nitrogen introduced were obtained when cultivating *F. braunii* than *L. multiflorum*, and in both cases, the introduction of magnesium fertilization reduced the values of these increases.

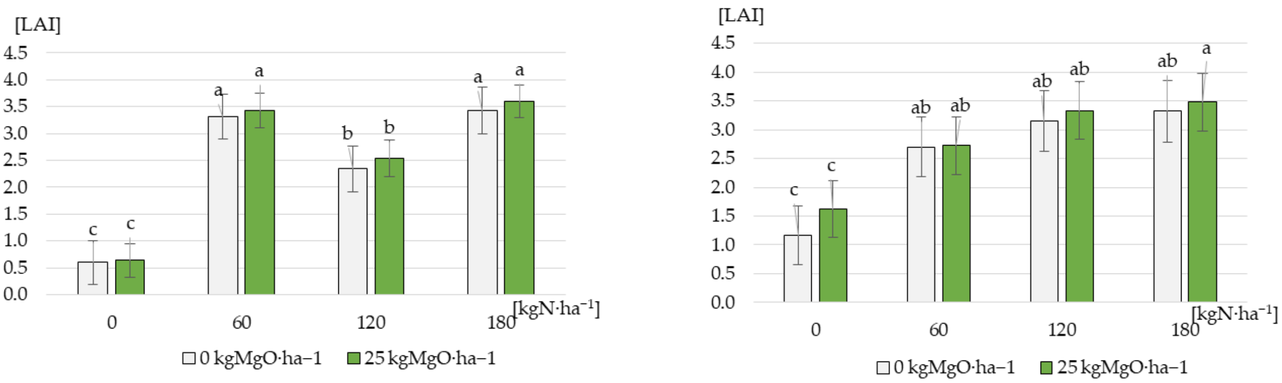
Table 1. Model equation of estimated expected values of maximum dry matter yields of plants fertilized with nitrogen within the limits of the tested doses.

Yield	*Festulolium braunii*			*Lolium multiflorum*		
	y [1]	**Max** [2]	**Dose** [3]	**y**	**Max**	**Dose**
Total (−Mg) [4]	$y = -0.0016x^2 + 0.57x + 46.79$ ($R^2 = 0.98$)	98.0	178.9	$y = -0.0025x^2 + 0.64x + 46.9$ ($R^2 = 0.99$)	88.6	129.2
Total (+Mg) [5]	$y = -0.0012x^2 + 0.49x + 51.12$ ($R^2 = 0.99$)	102.4	206.8	$y = -0.0017x^2 + 0.55x + 48.6$ ($R^2 = 0.96$)	93.8	163.0
1st cut (−Mg)	$y = -0.0018x^2 + 0.54x + 30.47$ ($R^2 = 0.97$)	72.2	152.2	$y = -0.0026x^2 + 0.61x + 34.52$ ($R^2 = 0.99$)	70.8	118.2
1st cut (+Mg)	$y = -0.0013x^2 + 0.47x + 35.39$ ($R^2 = 0.99$)	78.7	182.6	$y = -0.0017x^2 + 0.52x + 36.03$ ($R^2 = 0.96$)	77.2	155.5
2nd cut (−Mg)	$y = 0.056x + 15.71$ ($R^2 = 0.97$)	increase by 5.6 kg·1 kg N^{-1}		$y = 0.0362x + 12.32$ ($R^2 = 0.99$)	increase by 3.62 kg·1 kg N^{-1}	
2nd cut (+Mg)	$y = 0.0407x + 15.29$ ($R^2 = 0.98$)	increase by 4.07 kg·1 kg N^{-1}		$y = 0.0358x + 12.35$ ($R^2 = 0.96$)	increase by 3.58 kg·1 kg N^{-1}	

[1] y—expected value, [2] max—maximum yield (dt·ha^{-1}), [3] dose—dose (kg N·ha^{-1}), R^2—coefficient of determination, [4] (−Mg)—without Mg fertilization, [5] (+Mg)—with Mg fertilization.

3.2. Leaf Area Index and Chlorophyll Content

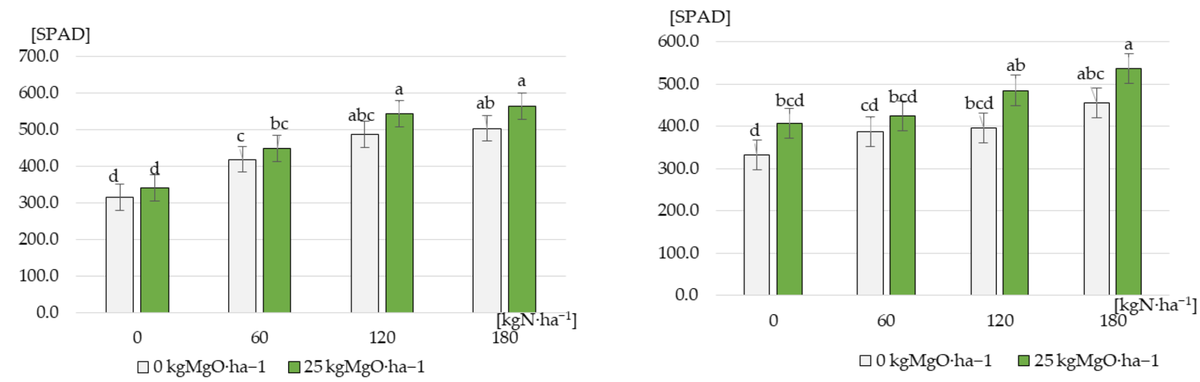
L. multiflorum plants were characterized by a higher LAI than *F. braunii* plants (Tables S3 and S4). In the first cut, the difference in the LAI index results between the compared species was 9%, and in the second cut, as much as 23%. The highest measured value of the LAI, amounting to 4.2, was found in *F. braunii* in the first cut in 2013 at a dose of 180 K_gN_{ha}. The experiments carried out for both grass species harvested in the first cut showed a clear tendency for magnesium and nitrogen fertilization to cooperate in influencing the LAI value (Figure 3). In *F. braunii*, the application of magnesium with nitrogen resulted in a tendency to obtain higher LAI index values, which, depending on the nitrogen dose, ranged from 0% for the not fertilized with N to 8.7% after the application of 120 K_gN_{ha} (Figure 3a). In the case of *L. multiflorum*, the effect of magnesium on increasing the leaf area before the first cut was recorded in nitrogen-fertilized combinations only in the case of doses of 120 and 180 K_gN_{ha}, and the increase in the LAI index value was 6.4% and 6.1%, respectively, and was statistically insignificant (Figure 3b).



(**a**) (**b**)

Figure 3. Leaf area index of (**a**) *Festulolium braunii* and (**b**) *Lolium multiflorum* plants measured before the first cut (LAI) in response to the interaction of Mg and N fertilization; on average in 2013–2014; letters denote homogeneous groups in Duncan's test ($p < 0.05$).

Similarly, in the case of chlorophyll content measured as SPAD values before the first cut, an interaction between magnesium and nitrogen fertilization in influencing the leaf greenness index values was proven (Figure 4). In each variant of nitrogen fertilization of both species, the use of additional magnesium fertilization led to an increase in the SPAD values, which were only trends within individual nitrogen doses. Statistically significant differences were obtained between doses of this fertilizer. For *F. braunii*, the differences in SPAD values between the objects fertilized with magnesium and those not fertilized with Mg increased with the increase in the nitrogen dose and, depending on the dose, ranged from 25.3 (0 K_gN_{ha}) to 60.6 (180 K_gN_{ha}) (Figure 4a). In turn, for *L. multiflorum*, the smallest difference between the object fertilized with magnesium and the unfertilized one was obtained for 60 K_gN_{ha} (37.5 SPAD), and the largest after applying magnesium at 120 K_gN_{ha} (88.4 SPAD) (Figure 4b). *F. braunii* plants were characterized by a slightly higher leaf greenness index than *L. multiflorum*, and their average values were 397.0 and 385.1 SPAD, respectively. The difference in favor of *F. braunii* was determined by the readings taken before harvesting the first cut, because in the measurements taken before the second cut, the SPAD values for both species were very similar (Table S5).



(**a**) (**b**)

Figure 4. Chlorophyll content of (**a**) *Festulolium braunii* and (**b**) *Lolium multiflorum* plants measured before the first cut (LAI) in response to the interaction of Mg and N fertilization; on average in 2013–2014; letters denote homogeneous groups in Duncan's test ($p < 0.05$).

3.3. Content of Nutrients in Plant Material

3.3.1. Chlorophyll a + b and Carotenoids

The Chl_a, Chl_b and sum of Chl_{a+b} content determined in leaf blades before the first cut was higher in *F. braunii* plants than in *L. multiflorum* plants, while in the analyses performed before the second cut, the results were contrary (Tables S6 and S7). There was no significant effect of magnesium fertilization on the Chl_a, Chl_b, Chl_{a+b} and Car content in the leaves of any of the grass species. Nitrogen fertilization significantly differentiated the concentration of Chl_a, Chl_b, Chl_{a+b} and Car in a first cut both in *Festulolium* and *Lolium* sp. It was shown that the increase in the Chl_a content, as a result of fertilization in *F. braunii*, was at doses of 60, 120 and 180 K_gN_{ha}, respectively: 0.37, 0.63 and 0.86 $mg{\cdot}g^{-1}$ FM, and for *L. multiflorum*: 0.43, 0.80 and 1.17 $mg{\cdot}g^{-1}$ FM, respectively, compared to an unfertilized variant. In *F. braunii* cultivation, the content of Car in leaf blades of the first cut increased from 4.00 in the control to 7.70 $mg{\cdot}g^{-1}$ FM at a dose of 180 K_gN_{ha}. The introduction of the highest nitrogen dose tested in *Lolium* cultivation also resulted in a significant increase in the content of Car in the leaves of the first cut and this increase amounted to 2.44 $mg{\cdot}g^{-1}$ DM compared to the control (Tables S6 and S7).

3.3.2. Nitrogen and Protein

F. braunii plants accumulated on average more nitrogen and protein in dry matter (11.4 $g{\cdot}kg^{-1}$ dry matter, 7.98%, respectively) than *L. multiflorum* (10.4 $g{\cdot}kg^{-1}$ dry matter, 7.2%, respectively) (Tables S8–S11). Nitrogen fertilization in the two grass species studied, using higher doses, caused an increase in the nitrogen content in the dry matter of plants, as well as the protein content in each year of the experiments and in both cuts. The highest nitrogen concentration was always obtained at a dose of 180 K_gN_{ha}, but in no case did the synthesis from years of research prove a difference between the concentration of this nutrient in plants after fertilizer application in doses of 120 or 180 K_gN_{ha} (Tables S8 and S9). Similarly, in each of the years of the study, for both grass species and their cuttings, an increase in protein concentration was shown with increasing nitrogen doses (Tables S10 and S11).

3.3.3. Nitrates and Nitrate Reductase in Forage

In our experiment, after applying each dose of nitrogen, the nitrate content in the forage from the first cuts of both grass species was safe for animals. The forage from *F. braunii*, in all fertilization variants, contained more nitrates than that obtained from *L. multiflorum* (Table S12). The content of nitrate reductase in plants of the first cut of *F. braunii* in 2013 was higher than in *L. multiflorum*, while in 2014 in the second cut, the effect was opposite. The introduction of magnesium fertilization did not have a significant impact on the content of nitrate reductase in the studied grass species, and the trends were undirected. On average, in 2013–2014, the reductase content in plants of the first and second cut increased as the nitrogen dose increased (Tables S13 and S14).

3.3.4. Crude Fat and Fiber

F. braunii plants in each cut contained more crude fat than *L. multiflorum*, and the difference for the first cut was 0.168% points and for the second cut, 0.265% points. The magnesium and nitrogen fertilization used in the experiments did not significantly differentiate the crude fat content in *F. braunii* and *L. multiflorum* plants (Table S15). The dry matter of plants of the first cut of *F. braunii* contained more NDF, ADF, cellulose and hemicellulose than in *L. multiflorum*, and the differences were 1.5, 2.28, 5.06 and 2.21% points. In turn, the content of the ADL fiber fraction was higher by 0.64% points in *L. multiflorum* (Tables S16–S19).

3.3.5. Macroelements

The dry matter of the first cut of *F. braunii* plants contained a lower amount of Ca (by 2.14 $g \cdot kg^{-1}$ dry weight) than that of *L. multiflorum*. The *F. braunii* plants of the first cut exceeded the *L. multiflorum* plants in the content of Mg, P and K by 0.07, 0.13 and 1.95 $g \cdot kg^{-1}$ DM, respectively. In turn, the plant material from the second cut of *F. braunii* was better than *L. multiflorum* only in terms of P content (by 0.05 $g \cdot kg^{-1}$ DM), and it contained more Ca, Mg and K by 0.97, 0.25 and 4.09 $g \cdot kg^{-1}$ DM, respectively. The use of increasing doses of nitrogen significantly varied the content of Mg and P in plants of the first cut of *F. braunii*. After the introduction of 120 and 180 K_gN_{ha}, a significant increase in Mg content was observed compared to the control, in both cases by 0.2 $g \cdot kg^{-1}$ DM. In turn, the proven increase in P content occurred up to a dose of 120 K_gN_{ha}, and compared to the control, it amounted to 0.5 $g \cdot kg^{-1}$ DM. A tendency to accumulate larger amounts of Ca in the plants of the 1st cut of *F. braunii* was observed with an increase in the nitrogen dose from 0 to 180 K_gN_{ha}. This tendency, however, only up to a dose of 120 K_gN_{ha}, also occurred in terms of P content. The content of any of the macroelements determined in the dry matter of *L. multiflorum* plants did not change significantly under the influence of fertilization (Tables S20 and S21).

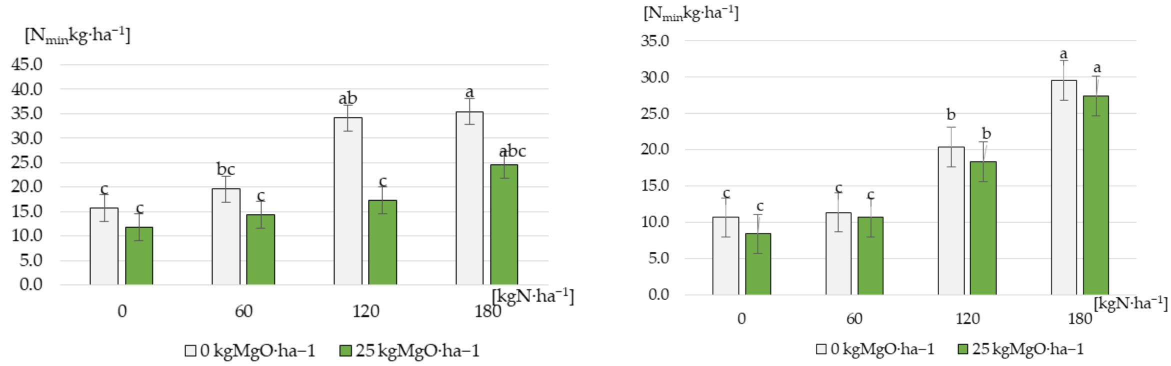
3.3.6. Forms of N Mineral Content in Soil after Harvest

After the first cut harvest of *F. braunii*, the amount of both forms of mineral nitrogen in the soil layer 0–30 was 9.6 $kg \cdot ha^{-1}$ higher than after *L. multiflorum* harvest (Table 2). In turn, after the second cut harvest, it was opposite, but the differences in these contents were smaller. The introduction of magnesium fertilization reduced the content of both forms of nitrogen in the soil after the first cut of both examined grass species, but differences were confirmed only for *F. braunii*. As expected, increasing the nitrogen dose led to an increase in the concentration of mineral nitrogen in the soil, more intensively under the cultivation of *F. braunii* than under *L. multiflorum*.

The use of magnesium fertilization together with nitrogen fertilization led to a decrease in the content of the ammonium form of nitrogen (NH_4) in the soil layer 0–30 cm after the first cut of *F. braunii* plants, significant after the introduction of 120 K_gN_{ha}. Such an effect reducing the burden on the soil environment has not been proven in the case of *L. multiflorum* (Figure 5).

Table 2. The content of individual forms of mineral nitrogen (N_{min} kg·ha^{-1}) in soil layers 0–30 cm after harvesting *Festulolium braunii* (*F. b.*) and *Lolium multiflorum* (*L. m.*), on average in 2013–2014; letters denote homogeneous groups in Duncan's test ($p < 0.05$).

Fertilization [kg·ha^{-1}]		**N-NH_4**				**N-NO_3**				**Total NH_4NO_3**			
		F. b.		***L. m.***		***F. b.***		***L. m.***		***F. b.***		***L. m.***	
		Cut				**Cut**				**Cut**			
		1st	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**
MgO	0	26.2 a	17.9 a	18.0 a	17.9 a	15.4 a	4.5 a	6.5 a	4.9 a	41.6 a	22.4 a	24.5 a	22.9 a
	25	17.0 b	16.0 a	16.8 a	19.2 a	8.6 b	4.2 a	6.7 a	5.1 a	25.6 b	20.2 a	23.5 a	24.2 a
N	0	13.8 b	15.1 b	9.6 c	17.3 b	6.2 a	4.0 a	3.2 b	3.4 b	20.0 b	19.0 b	12.8 c	20.6 b
	60	17.0 ab	16.2 ab	11.0 c	18.4 ab	8.4 a	4.2 a	4.6 b	5.0 ab	25.4 ab	20.5 ab	15.6 c	23.5 ab
	120	25.7 ab	16.9 ab	19.3 b	18.7 ab	15.2 a	4.4 a	7.8 ab	5.6 a	40.9 ab	21.4 ab	27.2 b	24.3 a
	180	30.0 a	19.5 a	29.8 a	19.9 a	18.0 a	4.8 a	10.7 a	6.0 a	48.0 a	24.3 a	40.4 a	25.9 a
Average 2013–2014		21.6	16.9	17.4	18.6	12.0	4.4	6.6	5.0	33.6	21.3	24.0	23.6



(**a**) (**b**)

Figure 5. Content of the ammonium form of nitrogen in the soil layer 0–30 cm after the 1st cut of (**a**) *Festulolium braunii* and (**b**) *Lolium multiflorum* plants in response to the interaction of Mg and N fertilization; on average in 2013–2014; letters denote homogeneous groups in Duncan's test ($p < 0.05$).

3.4. Nitrogen Accumulation and Indices of Efficiency

Nitrogen uptake by plants of both grass species harvested in the first cut was significantly higher than in the second cut, and *F. braunii* plants accumulated more nitrogen than *L. multiflorum*, especially in the second cut. The introduction of magnesium fertilization alongside nitrogen fertilization increased nitrogen uptake by plants of both species harvested in the first cut. Moreover, increasing the nitrogen dose introduced in both grasses led to a systematic increase in the uptake of N by plants harvested in both cuttings (Table 3).

In *F. braunii* cultivation, magnesium fertilization did not significantly increase the agricultural efficiency of the nitrogen applied at doses of 120 and 180 K_gN_{ha}. A clear tendency to improve this efficiency as a result of the introduction of magnesium fertilization was observed in *L. multiflorum* cultivation and it was statistically confirmed at a dose of 180 K_gN_{ha} (Figure 6a). In turn, magnesium fertilization significantly reduced the physiological effectiveness of nitrogen use in *F. braunii*, while in *L. multiflorum*, this effect was the opposite (Figure 6b). In general, however, it can be noticed that increasing the nitrogen dose resulted in a decrease in the agricultural and physiological efficiency of nitrogen use (Figure 6).

Table 3. Nitrogen uptake by plants depending on the fertilization used (K_gN_{ha}), on average in 2013–2014; letters denote homogeneous groups in Duncan's test ($p < 0.05$).

Fertilization		*Festulolium braunii*		*Lolium multiflorum*	
		1st [1]	2nd	1st	2nd
MgO [kg·ha^{-1}]	0	62.7 a	25.1 a	60.2 a	16.2 a
	25	68.3 a	22.4 a	64.4 a	16.8 a
N [kg·ha^{-1}]	0	23.8 d	17.7 c	28.8 c	11.9 c
	60	59.0 c	20.9 bc	60.8 b	15.1 bc
	120	82.3 b	25.5 ab	78.8 a	17.4 b
	180	97.0 a	30.8 a	80.7 a	21.5 a
Average 2013–2014		65.53	23.73	62.28	16.48

[1] time of cut.

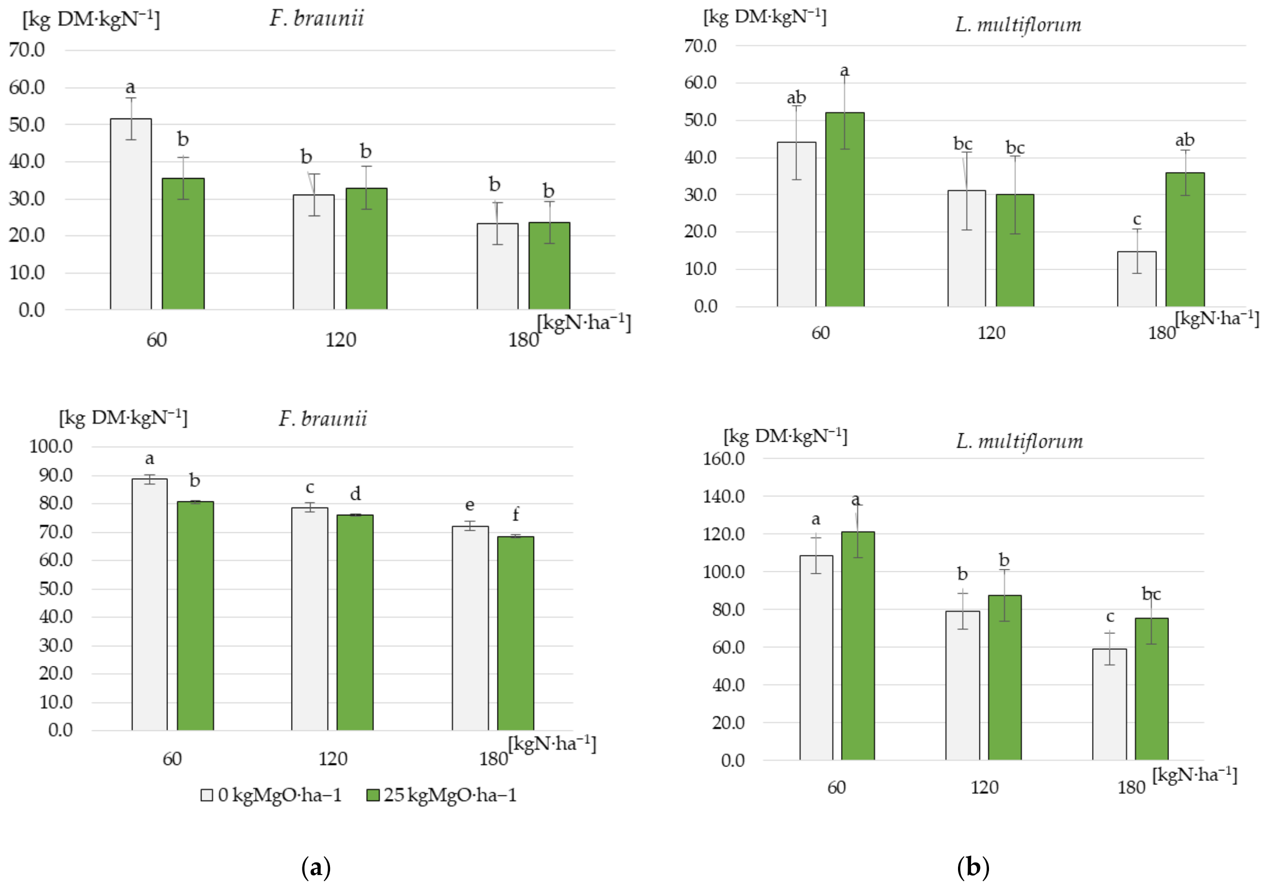


Figure 6. Agricultural (**a**,**b**) physiological effectiveness of nitrogen use in the 1st regrowth of *Festulolium braunii* and *Lolium multiflorum* plants in response to the interaction of Mg and N fertilization; letters denote homogeneous groups in Duncan's test ($p < 0.05$).

The content of mineral nitrogen (NH_4NO_3) in the soil of the 0–30 cm layer and calculation of the potential DM yield of the second cut of both grass species showed that after applying each of the tested N doses, the availability of nitrogen in the soil was a factor not limiting the yield of plants harvested in the second cut. It was shown that the deficiency of rainfall during the second part of the plant vegetation allowed the use of nitrogen contained in the soil to produce 54.8–89.6% of the potential yield of the second cut (Table 4).

Table 4. Theoretical yield of the 2nd cut calculated on the basis of the N_{min} content in the soil of the 0–30 cm layer.

Dose K_gN_{ha}	NH_4NO_3 in Soil N_{min} $kg \cdot ha^{-1}$	DM Yield 1st Cut $t \cdot ha^{-1}$	N Uptake K_gN_{ha}	Unit Uptake [1] kg $N \cdot t^{-1}$	DM Yield 2nd Cut $t \cdot ha^{-1}$		
					Theoretical [2]	Actual	Relatively%
				Festulolium braunii			
60	25.4	5.88	59.0	10.03	2.53	1.77	70.0
120	40.9	7.10	82.3	11.59	3.53	2.10	59.5
180	48.0	7.48	97.0	12.97	3.70	2.46	66.5
				Lolium multiflorum			
60	15.6	6.36	60.8	9.56	1.63	1.46	89.6
120	27.2	7.14	78.8	11.04	2.46	1.62	65.8
180	40.4	6.94	80.7	11.63	3.47	1.90	54.8

[1] Unit uptake (N uptake/DM yield), [2] theoretical DM yield 2nd cut (NH_4NO_3 content/unit uptake).

3.5. Metabolizable Energy

L. multiflorum was characterized by a higher metabolizable energy content in DM for horses than *F. braunii*, and the difference was 0.35 $MJ \cdot kg^{-1}$ DM. Magnesium fertilization had no effect on the content of this feed quality parameter in the case of *F. braunii*, while in the case of *L. multiflorum* in the N with Mg fertilization variant, it was determined by 0.22 $MJ \cdot kg^{-1}$ DM less metabolizable energy. When differentiated nitrogen fertilization was used, the best effect was achieved for both grass species at a dose of 60 K_gN_{ha}. Increasing nitrogen fertilization doses did not result in an increase in the metabolizable energy content calculated for horses in the tested grass species.

The yield of metabolizable energy calculated for horses was higher from the first cut of *L. multiflorum* (20.43 $GJ \cdot ha^{-1}$) than that of *F. braunii* (18.23 $GJ \cdot ha^{-1}$). The introduction of magnesium fertilization in the case of both grass species resulted in an increase in the yield of metabolizable energy, which for *F. braunii* and *L. multiflorum*, amounted to 0.95 and 0.31 $GJ \cdot ha^{-1}$, respectively. Nitrogen fertilization of *F. braunii* led to a systematic increase in the yield of metabolizable energy, with an increase in the applied dose, from 10.74 $GJ \cdot ha^{-1}$ at a dose of 0 K_gN_{ha} to 22.46 $GJ \cdot ha^{-1}$ at a dose of 180 K_gN_{ha}. In the case of *L. multiflorum*, the increase in the metabolic energy yield of the first cut occurred only up to the dose of 60 K_gN_{ha}, and compared to the control object, it amounted to 12.56 $GJ \cdot ha^{-1}$ (Table 5).

Table 5. Metabolizable energy for horses and its yield in dry matter of the 1st cut of *Festulolium braunii* and plants of *Lolium multiflorum* depending on the fertilization used; average 2013–2014; letters denote homogeneous groups in Duncan's test ($p < 0.05$).

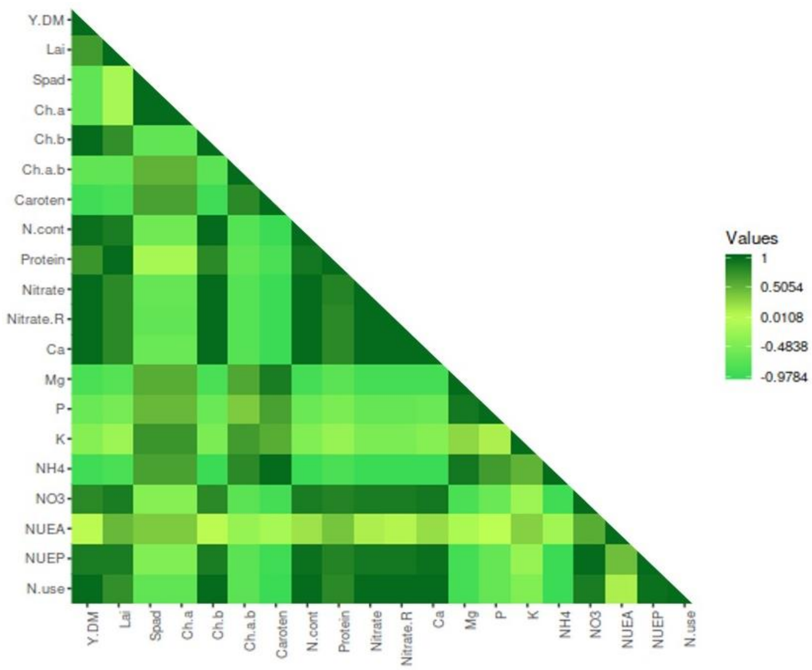
Fertilization		*Festulolium braunii*		*Lolium multiflorum*	
		EM [1]	Ym [2]	EM	Ym
MgO [$kg \cdot ha^{-1}$]	0	3.11	17.68	3.53	20.26
	25	3.01	18.63	3.31	20.56
N [$kg \cdot ha^{-1}$]	0	3.29	10.74	3.51	12.18
	60	3.06	18.04	3.89	24.74
	120	2.97	21.10	3.41	24.33
	180	3.00	22.46	2.87	19.92
Average 2013–2014		3.07	18.23	3.42	20.43

[1] metabolizable energy [$MJ \cdot kg^{-1}$ DM], [2] yield of metabolizable energy [$GJ \cdot ha^{-1}$].

3.6. Data Visualization and Analysis—Relationship among Mg and N Dose Treatments and Observed Parameters

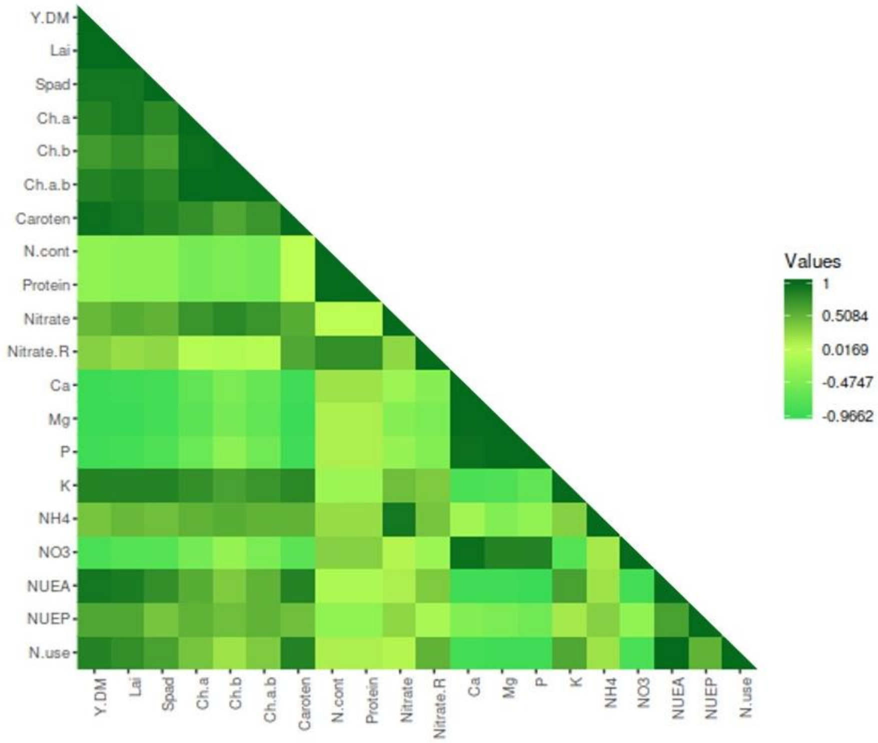
A heatmap with dendrograms was used to analyze and visualize the data from the study (Figure S1). The dendrograms sort the treatments used in our study (Mg 0 and 25 $kg \cdot ha^{-1}$, N 0, 60, 120 and 180 $kg \cdot ha^{-1}$), as well as the parameters studied (dry matter yield, content of nutrients in plant material, forms of N mineral content in soil after harvest, nitrogen accumulation and indices of efficiency) according to their similarity. In *F. braunii* plants, the lateral dendrogram reveals a similarity between the Mg0 + N0 and Mg25 + N0 treatment. Both the Mg0 + N0 treatments are grouped in a cluster together with the N0 + Mg25 treatment in 2013 and 2014. The heatmap shows that these treatments had mostly negative or no effects on the parameters studied. Similar dependencies were observed in *L. multiflorum* in 2013. Another cluster in *F. braunii* is formed by the treatments Mg0 + N60, Mg0 + N120, Mg0 + N180, Mg25 + N60, Mg25 + N120 and Mg25 + N180 in both first cuts of the two studied years, which share common features. The coloring clearly shows the increase in N accumulation, dry matter yield and decrease in N-NO_3 and N-NH_4 form. In *L. multiflorum* the same treatments formed a cluster in 2013. The similar increase in N accumulation and dry matter yield were observed, but opposite to *F. braunii* with an increase in N-NO_3 and N-NH_4 form.

Whereas the upper dendrogram of the heatmap indicates the relationships among parameters (Figure S1), the correlation matrix heatmap offers an insight into the correlation among them (Figure 7). In *F. braunii* plants, the strong positive correlation was detected between the dry matter yield with the N accumulation (0.98) and physiological nitrogen use efficiency (0.86). The dry matter yield showed further positive connections with Ca content (0.96) and N-NO_3 form (0.77) as well as with N content (0.94), protein content (0.97), nitrate reductase (0.97) and chlorophyll b content (0.98). In turn, N-NH4 form (−0.92) and K content (−0.41) correlate negatively with dry matter yield (Figure 7a). In *Lolium multiflorum* plants, the same as in *F. braunii*, correlation was detected between the dry matter yield and the N accumulation, but it was weaker (0.60). A strong positive correlation was detected between N and nitrate content (0.97) and between nitrate and Ca content (0.99).



(**a**)

Figure 7. *Cont.*



(**b**)

Figure 7. The correlation matrix heatmaps for *Festulolium braunii* (**a**) and *Lolium multiflorum* (**b**) show the values of the Pearson correlation coefficient for all studied parameters, the positive values are in dark green, negative are in light green. The coefficient ranges from −1 (perfect negative linear relationship) to 1 (perfect positive linear relationship), while the value 0 indicates that there is no relationship between studied variables. The parameters analyzed were Y DM—dry matter yield; Spad—chlorophyll content index; Lai—leaf area index; Chl_a—chlorophyll a; Chl_b—chlorophyll b; Chl_{a+b}—chlorophyll a + b; Caroten—carotenoids; N cont—nitrogen content; Protein—protein content; Nitrates—nitrates content in dry matter; Nitrate R—nitrate reductase; P—P content, K—K content; Ca—Ca content, Mg—Mg content; NO_3—N-NO_3 form; NH_4—N-NH_4 form; N use—N accumulation; NUEP—physiological nitrogen use efficiency; NUEA—agricultural nitrogen use efficiency.

4. Discussion

One of the main global problems in agriculture, due to climate change, is the production of sufficient amounts of animal feed. In many countries, prolonged drought periods in spring and summer have increased, and this has caused increasing interest in forgotten grass species, as well as the search for solutions in conventional plant breeding technologies, from which grass hybrids are the result. In recent years, more common use has been made of inter-generic hybrids created by crossing between species of the ryegrass (*Lolium*) and fescue (*Festuca*) genera called *Festuloliums* [37]. *Festulolium* cultivars combine the high forage nutritive value and digestibility of the *Lolium* parent with the superior resistance to abiotic stresses and edaphic and climatic adaptations of *Festuca* [38,39]. The varieties of *Festulolium* sp., both in Poland and Slovakia, compared to the varieties of *Lolium*, have turned out to be more durable and more fertile, as well as more resistant to unfavorable climatic conditions (low temperatures and droughts, especially in Poland). Our results are consistent with those reported by Ghesquière et al. [40], Yamada et al. [41], Fariaszewska et al. [42], Kitczak et al. [43], Humphreys et al. [44] and Nekrošas and Kemešytė [45]. Based on our research, it was found that *L. multiflorum* was characterized by lower yields compared to *F. braunii*, especially in the conditions of the second regrowth, which took place in the summer. In conditions of higher temperatures and sunlight in summer, *L. multiflorum* enables faster growth of plant biomass. According to Huot et al. [46], *Lolium* as C4 photosynthetic-type plants are characterized by rapid establishment and growth, which leads to weed control, as well as a very good capacity for winter survival and regrowth, which allows a high yield.

Festulolium combines high yields and good digestibility, two important factors in forage production [47,48]. One of the most important measures influencing the nutritional value of forage from grasslands is the content of total protein. In our own research, *F. braunii* plants on average accumulated more protein (11.4 $g \cdot kg^{-1}$ DM) than *L. multiflorum* (10.4 $g \cdot kg^{-1}$ DM), but exceptionally low values were obtained. The literature data show a wide range from 66 $g \cdot kg^{-1}$ DM to 194 $g \cdot kg^{-1}$ DM depending on environmental conditions and fertilization [43,49,50]. According to Staniak and Harasim [51], drought stress significantly reduced the content of crude fibre, and the contents of crude protein and crude fat tended to increase. The same average crude protein content in *Festulolium* and *Lolium* cultivars was determined in Babić et al.'s [52] studies, but the authors noted that having in mind the fact that in the total dry matter yield in perennial grasses, the first cut makes up more than 70%, *Festulolium* can be a reliable source of sufficient quantities of quality fodder. Similarly to crude protein content, ADF and NDF content in forage grasses, as well, depends on the environment where forage grasses are grown [52]. Too high a fibre content worsens the digestibility of the forage [43]. In our experiments, all fiber fractions, except ADL, as well as hemicellulose, cellulose and crude fat were higher in *Festulolium* than in *Lolium*. According to Kotlarz et al. [53], NDF content in forage is strictly dependent on the harvest data and, especially, on thermal conditions. Our results were higher in comparison to Wiśniewska-Kadżajan and Stefaniak [54] and, at the same time, were generally higher than that recommended by Mertens [55]. In turn, ADF fraction values were comparable to the results obtained by the above authors and it complied with the feed requirements provided by Brzóska and Śliwiński (2011) [56]. *F. braunii* plants in the first growth were characterized by higher concentrations of magnesium, phosphorus and potassium compared to *L. multiflorum* by 0.08, 0.13 and 1.95 $g \cdot kg^{-1}$ dry matter, respectively, but the values of Mg, P and K content in *F. braunii* plants were in accordance with those obtained in Sosnowski and Truba's [57] studies.

There is no doubt that nitrogen supply is of particular importance; it determines the yield and contributes to an increase in total and digestible protein content. Nitrogen availability is considered as a factor that has been known to increase resistance and resilience towards drought stress [58]. It can be explained by a physiological effect on the plants' water regulatory system, as N fertilization in our studies leads to increased protein contents and thus higher concentrations of Rubisco in the plant tissue, enabling plants to close their stomata sooner and conserve water during drought [59]. We assumed that this direct effect of N $\times$ Mg fertilization has also increased the drought resistance of two grass species, though not tested in this study. The results leads us to the conclusion that our fertilization regime as well as our grass management was fit to the site, using slow-release fertilizers that have the potential to provide a nutrient release pattern that aligns with plant requirements [60]. The simulation studies showed the release time of nutrients from such fertilizers is at least three months [61], which could explain the fact that N was available in the soil for the second cut, so our hypothesis including the one-time application of nitrogen fertilization, applied in the form of nitrate and urea after the start of vegetation, was proved. However, the majority of recommendations suggested splitting the N dose, while Redfearn et al. [62] noticed that for dryland bermudagrass hay production, a single N application of 200 pounds N per acre in early May appears to be adequate and that there is usually no benefit to split N applications in bermudagrass due to variable rainfall distribution that can occur during the season. There is still a lack of such research on the assessment of nitrogen input under summer drought conditions.

Magnesium is a frequently forgotten element with a well-known fundamental role in plant metabolism, but the number of studies addressing the significance of Mg for the quality of agricultural produce still appears very limited as compared to other major nutrients [63]. Gutmane and Adamovich [64] concluded from their studies that dry matter yield of *Festulolium* and hybrid ryegrass was found to be strongly dependent on the weather conditions in each year of the trial, and particularly so during the periods of summer regrowth. Similarly, the different spring weather patterns allowed us to observe the significant impact of nitrogen fertilization on the vitality and yield of grass plants. This was particularly visible when the factor limiting yield was water shortage and unfavorable rainfall distribution. The regression equation showing the relationship between the total dry yield of *F. braunii* and nitrogen fertilization in the dose range of 0–180 K_gN_{ha} had a parabolic course. The highest yield of dry matter for this species could be harvested after the application of 191.1 K_gN_{ha} and it would have amounted to 102.2 dt·ha^{-1}. In turn, the maximum yield of the first cut of *F. braunii* (74.05 dt·ha^{-1}) and *L. multiflorum* (74.22 dt·ha^{-1}) could be expected after contributing 159.8 and 136.2 K_gN_{ha}, respectively. Such N doses are in accordance with the recommendations under more sustainable agricultural practices, indicating that the N fertilizer rate has to be reduced to an ecological optimum in the order of 150–200 kg·ha^{-1} per year [65]. In Mastalerczuk et al.'s [66] experiment, the influence of the N dose on two *Festulolium* varieties' (including 'Felopa') biomass was visible in the last period of study in which the total plant biomass was the smallest with no N application in relation to fertilized plants with a single dose of 90 K_gN_{ha} and double. In turn, Borowiecki [67] found that the highest yield of dry matter was obtained at the nitrogen fertilization rate of 240 K_gN_{ha}, divided after the first, second and third cut.

Although the literature findings are limited, Wang et al.'s [68] analysis suggested that Mg application improved grass yield by 10.6%, but also, in general, the yield increase in crops was 10.6% under severe Mg deficiency and 10.8% when soil pH was lower than 6.5. In our studied years, under conditions of average Mg content in the soil, plants of both species responded with an increase in the total yield by 3.9 (*F. braunii*) and 6.05% (*L. multiflorum*). The significant interaction of N and Mg fertilization in our studies proved that the supply of nitrogen to a plant besides numerous factors, depends on magnesium. One of the results of low magnesium supply is the time-limited transport of assimilates to roots that results as the main cause of slow growth of the whole root system, in turn decreasing its capacity for water and nutrient uptake [69,70], so the rate of growth and final size of leaves can be limited [71]. In terms of the rate of canopy growth measured as the leaf area index (LAI), when reaching the level of 3.0 (on sugar beet example), it is suggested that the plant efficiently exploits solar radiation, which depends on the leaf surface area, driven by the nitrogen supply [72]. In our studies, the LAI reached on average values of 2.47 (*F. b.*) and 2.68 (*L. m.*), but nitrogen fertilization significantly increased this level to 3.4 in *F. braunii* and *L. multiflorum* at doses of 60 and 180 K_gN_{ha}, respectively. The use of Mg increased the level of the LAI in two grass species, but the effect was not significant. The literature indicates a positive correlation between N content and SPAD readings [73], as well as between chlorophyll content in the leaf blade of grasses and its dry matter yield [74]. Our results indicated that the values of the SPAD index increased under a higher N dose, similarly to that in Mastalerczuk et al. [66], in which *F. braunii* 'Felopa' measured on average 37.0 SPAD units. In our experiment, the same variety reached 514.8 SPAD values at a dose of 120 K_gN_{ha}. Such discrepancies may result from the fact that the authors' pot experiment was conducted in greenhouse conditions and nitrogen fertilization was lower. We observed the increased value in SPAD under Mg fertilization only in the first cut of *L. multiflorum*, which was consistent with Sun et al.'s [75] studies.

Under certain conditions, such as high nitrogen fertilization, drought or abrupt changes in weather, plants can develop dangerously high nitrate levels. The main problem with excess nitrates is that they are converted in the rumen to nitrites that are absorbed in the bloodstream and ultimately prevent the red blood cells from carrying life-giving oxygen. Death or abortion may result as a consequence of nitrate intoxication [76]. The safe limit value for nitrate content in forage for horses is up to 10 g NO_3/100 kg body weight according to Cockburn et al. [77]. The nitrate content in the forage from the first cuts of both grass species was safe for animals when applying each dose of nitrogen. Nitrate content may be dependent not only on N fertilization but also species, which may be more susceptible to nitrate accumulation [78], while Algan and Aydin [79] proved that different forages had increased toxic levels in accordance with increasing rates of N fertilizer independent of the type of forage.

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents have been taken into account to assess roughage quality and are commonly used to calculate the amount of feedstuff an animal is able to digest, total digestible nutrient content and other energy components [49]. NDF constitutes a source of energy for rumen microorganisms, gives the feed a structure and serves as ballast filling the rumen [56]. It is widely used instead of raw fibre in the modern animal nutrition system [80]. The average values of NDF and ADF content in *F. braunii* were similar to those obtained in Malinowska and Jankowski's [49] studies, but opposite to ours, where fiber fraction contents increased in grass with increasing N and a biostimulant. In turn, Jankowska [81] reported that increasing nitrogen doses reduced the share of the ADF fraction in meadow hay. The reduction in its amount improves forage digestibility; thus, its content is a good indicator of roughage quality [82]. The average ADF contents in *F. braunii* and *L. multiflorum* amounted to 309.0 and 378.2, respectively, with no changes in N fertilization. Values are within the range given by Brzóska and Śliwiński [56] and Grzelak and Bocian [83]. An increase in ADF content was observed by Malinowska and Jankowski [49] in *F. braunii* as an effect of nitrogen applied, but these differences were not statistically significant.

The variability in forage protein in response to Mg fertilizer largely depends upon the available N [75,84,85]. In each of the years of our studies, for both species of grass and their cuttings, an increase in the concentration of total protein was shown with increasing nitrogen doses. There was no interaction between magnesium and nitrogen fertilization. Magnesium fertilization in our studies increased protein content in *L. multiflorum*, while Sun et al. [75] showed the opposite effect in annual ryegrass leaves, where crude protein at all levels of Mg addition was significantly decreased, which was explained by the low content of N in the soil.

An excessive concentration of mineral nitrogen in soil constitutes a potential threat for the natural environment, so attempts are made to improve the effectiveness of fertilization with this nutrient. As per our hypothesis, ammonia (N-NH_4) and nitrate (N-NO_3) forms were significantly modified by the dose of magnesium, especially in *F. braunii* cultivation. Similarly, some previous studies on maize showed significantly lower amounts of the nitrate form of mineral nitrogen in soil found for the object, where 25 K_gMgO_{ha} was applied in comparison with the object with no magnesium application [86].

Magnesium is considered as a crucial nutrient for the nitrogen uptake, especially nitrates, by high-yielding crop plants, due to the hydrogen pump efficiency, which requires high H^+-ATP-ase activity, depending in turn on Mg^{2+} as an enzyme cofactor [18], and simultaneously controlling processes responsible for photosynthesis and assimilate production and partitioning among plant parts [66]. Only a few new reports in soybean [87], rapeseed [88] and maize [89] have indicated the importance of magnesium in N uptake, while magnesium has still received little attention in terms of fertilization. In our two grass species tested, magnesium fertilization caused a slight increase in N uptake, but it was not statistically significant. The effect of interaction between magnesium and nitrogen fertilization in two grass varieties became more visible and could be named according to Grzebisz [90] as 'magnesium-induced nitrogen uptake' when comparing the results of the agricultural and physiological effectiveness of nitrogen use indicators. Our results, although undirected, showed more clearly the higher effectiveness of nitrogen use in the first regrowth of *L. multiflorum* plants under a higher N dose (180 K_gN_{ha}) with Mg fertilization.

5. Conclusions

In field cultivation carried out on a light soil under conditions of recurrent summer droughts, higher yields of dry matter are harvested from *F. braunii* 'Felopa' than *L. multiflorum* 'Tur' variety. In such conditions, it is justified to apply nitrogen once immediately after the start of vegetation in two forms: fast-acting nitrate (20 K_gN_{ha}) and slow-acting urea (supplementary dose). Resignation from splitting the nitrogen dose and using its full dose in spring, after the start of vegetation, instead of adding it also after the first cut during the dry summer, allows a reduction in the losses of this nutrient resulting from the volatilization of ammonia. Moreover, the burden on the soil environment is avoided because the mineral nitrogen content remaining in the soil is at a low level, and is easily utilized by microorganisms that decompose crop residues. The nitrate content in the forage used for horses resulting from a single nitrogen introduction is safe for animals, including the most sensitive horses. The introduction of magnesium fertilization alongside nitrogen fertilization in both grass species resulted in an increase in the maximum yield value, with a simultaneous increase in the nitrogen dose. The increase in actual yield was only a trend observed after the application of higher doses of N. A beneficial effect for the environment of such fertilization was a significant decrease in the concentration of mineral nitrogen in the soil.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14051086/s1, Table S1: Dry matter yield of *Festulolium braunii* [dt·ha^{-1}]; Table S2: Dry matter yield of *Lolium multiflorum* [dt·ha^{-1}]; Table S3: The leaf area index *of Festulolium braunii* [LAI]; Table S4: The leaf area index of *Lolium multiflorum* [LAI]; Table S5: Chlorophyll content [SPAD] of *Festulolium braunii* and *Lolium multiflorum*, average in 2013–2014; Table S6: Chlorophyll a, b, a + b and carotenoids content of *Festulolium braunii* [mg·g^{-1} FM], average in 2013–2014; Table S7: Chlorophyll a, b, a + b and carotenoids content of *Lolium multiflorum* [mg·g^{-1} FM], average in 2013–2014; Table S8: Nitrogen content of *Festulolium braunii* [g·kg^{-1} DM] plants; Table S9: Nitrogen content of *Lolium multiflorum* [g·kg^{-1} DM] plants; Table S10: Protein content of *Festulolium braunii* [g·kg^{-1} DM] plants; Table S11: Protein content of *Lolium multiflorum* [g·kg^{-1} DM] plants; Table S12. Nitrate content in dry matter of 1st cut of *F. braunii* and *L. multiflorum* depending on the fertilization applied, average in 2013–2014;Table S13: Nitrate reductase content of *Festulolium braunii* [nmole NO_2^-·g^{-1}·h^{-1}] plants; Table S14: Nitrate reductase content of *Lolium multiflorum* [nmole NO_2^-·g^{-1}·h^{-1}] plants; Table S15: Crude fat content [%] in *Festulolium braunii* and *Lolium multiflorum* plants; Table S16: Fiber fraction [%] in the dry matter of *Festulolium braunii* plants, 1st cut; average in 2013–2014; Table S17: Fiber fraction [%] in the dry matter of *Festulolium braunii* plants, 2nd cut; average in 2013–2014; Table S18: Fiber fraction [%] in the dry matter of *Lolium multiflorum* plants, 1st cut; average in 2013–2014; Table S19: Fiber fraction [%] in the dry matter of *Lolium multiflorum* plants, 2nd cut; average in 2013–2014; Table S20: Macroelements content in the dry matter of *Festulolium braunii* plants [g·kg^{-1} DM]; Table S21: Macroelements content in the dry matter of *Lolium multiflorum* plants [g·kg^{-1} DM]; Figure S1: The heatmap analysis of the parameters studied in the treatments.

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